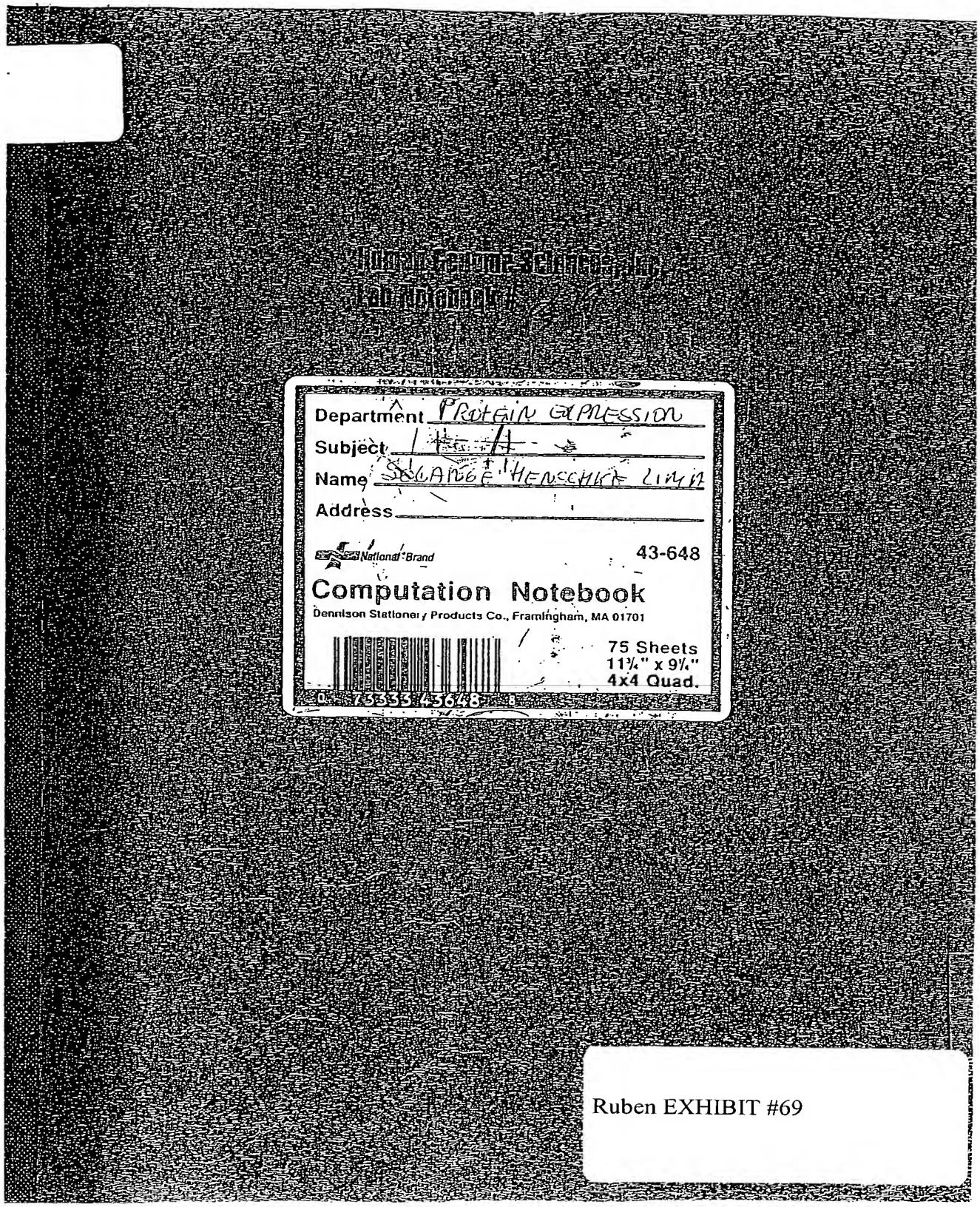


Ruben EXHIBIT #69

Department PROTEIN EXPRESSION

Subject

Name SUZANNE HENSCHKE LIMA

Address

National Brand 43-648

Computation Notebook

Dennison Stationery Products Co., Framingham, MA 01701

75 Sheets
11¾" x 9¼"
4x4 Quad.

≠
1 A.a 30 µm ⇒ split to 60 µm m+x
1 A.a 60 µm ⇒ " to 70 µM and MEM d+

≠ 1 A.b 50 µm ⇒ split to 60 µm
1 A.b 60 µM ⇒ split to 70 µm m+x and in MEM d+

≠ 1 A.d 50 µm ⇒ split to 60 µm
1 A.d 60 µm ⇒ split to 70 µm m+x and in MEM d+

2/17/95 Injections Spinner Flasks in EX-401 of RB3

REDACTED

2/17/95 Transfections in Sf9 cells

REDACTED

3) H+PANO8SO4 - 51 bp
4) H+PANO8SO4 - 185 bp

the DNA concentration of all were ≈ 1 µg/ml

#s 1, 2, 3, 4 I have the sequence report sheet

REDACTED

procedure like page 1

4

2/21/95 2 gave ~500 ml of supernatant to Mark

REDACTED

2/22/95 PLAQUE PURIFICATION:

REDACTED

HTPAN08S04-S1bp

HTPAN08S04-185bp

REDACTED

2 22 95.

REDACTED

2 22 95

2 22 95 2 harvested the transfections in Sf9 cells from 2.17.95

REDACTED

3) HTPAN08 SO4 - S1 bp

4) HTPAN08 SO4 - 1856 bp

REDACTED

REDACTED

3.1.95 Injection S/S cells with plaque purified virus of

REDACTED

HTPAN08504 - S1bp

* HTPAN08504 - 125bp

from 2.22.95

2 plaques of each

* 1 had plaques only in the first dilution

REDACTED

3.6.95 Harvested S19 cells infected on 3.1.95 with plaque purified lines
blue assay

REDACTED

HtPAN08504 (-1856p) ① +++
HtPAN08504 (-1856p) ② ++

HtPAN08504 (-515p) ① - (negative) ⇒ to be redone
HtPAN08504 (-515p) ② extremely weak ⇒ redone
↓
Xept

D.obs: redone on 3.8.95

REDACTED

3/8/95 Injection of 19 cells with plaque purified virus of

REDACTED

3) HTPAV0850A-S16D from 2.2.95

REDACTED

3/2/95 Split CHO Staurocalcin clones from 3.1.95

all 4 clones are finished and amplified up to 100 µM Mtx and the cells were used today in MEM1+

Stc 7 1 A.d
Stc 7 1 A e
Stc 7 1 B C
Stc 7 1 A b

3/3/95 Harvested transfections from 3.8.95

Drac 04-101 → did not work for the 2nd time!!

HPFCT89X (PS6-3) → blue assays positive!

3/3/95 Harvested Sf9 cells infected with plaque purified virus from 3.8.95

HIBCL22 ③ good
④ weaker

HUVEC101532 (VI6E) ① both good
②

HTPAN08-Sibp ⑤ ⑥ did not work for the 2nd time → has to be plaque purified again

3/3/95 NOTE: today Reiner send to Frank Hirschen 3 vials of FLAP, labelled as: V-HAPAK-1 = Flap 1 to 16
V-HAPAK-2 = Flap 1 to 14

18

3.14.95 Harvested the virus stock HTPAN0850 ÷ 185.bp
from 3.9.95 (not in the lab book)
the blue assay was positive

REDACTED

22

REDACTED

3 2375 Injections Spinner flasks in CX-01 PBS

REDACTED

1 4 HTPA ND8504:-18S bP (Fa, Ligand)

REDACTED

24

3 28 95 Harvested Spinners flasks Injected on 3.23.95

REDACTED

1 11 HtPAnD8504 -185bp → Supe and cells to Nagase

REDACTED

REDACTED

REDACTED

3.30.95 Injections Spinner flasks in CX-401 @ CBR

REDACTED

26

2 specimens HtPAN0804-185bp

REDACTED

REDACTED

4/5/95 Harvested the spinner flasks inspected on 3/30/95

REDACTED

HIPAN08504-185P to Rajeev

REDACTED

REDACTED

9/11/95	PLAQUE PURIFICATION
	HtPAN08504 -S1bP Nohone

REDACTED

REDACTED

4.18.95 Injection Sf9 cells with plaque purified virus from 4.11.95

HTPAN08S04 - S16p - was contaminated!

REDACTED

see 4.21.95

I pick three plaques of each ... the plaques were to small!!

SUPERVISOR- [signature]
DATE- 4/19/95

[initials]

REDACTED

4.21.95	note: the Sf9 cells injected on 4.18.95 with plaque purified virus were not positive in the blue assay so I decided to purify them again
	⇓
4.21.95	PLAQUE PURIFICATION
	HTPAN08S04 - 56bp
	HFKEB40 6-4
	HD6RC10

J. [illegible]

REDACTED

4'21'95	Injection Lfs cells with plaque purified plaques from 4.21.95 HTPAN08504-S1 bp is small plaques

REDACTED

38

5 3 95 Harvested Sf9 cells injected with plaque purified virus from 4.27.95 blue assay according to Pam

HTPAV08504-S1, bP ① +

② +

REDACTED

5/5/15 Injection s/p cells for virus stock

HtPANO8S04 -s16p ②

REDACTED

Amount of virus: 50 ul/flask

REDACTED

HtPANO8S04 -s16p ②

REDACTED

blue assay positive for all!

REDACTED

5/19/95 thawed new SJ9 cells

1 vial of SJ9 3rd Grace 10% FBS - D.S. $\times 10^6$/ml P: 31-32

1 vial of SJ9 in ex-cell 21: FBS - D.S. 6×10^6/ml P: 31-32

Procedure:

- thaw the vials quickly in 37°C bath
- resuspend the cells and transfer them to a tube with 10 ml of media
- spin for 5' at 0.1 rpm
- discard the supernatant
- resuspend the pellet in 1 ml of media
- inoculate in 25 cm^2 T flask
- incubate at 27°C for 2 to 3 days

SUPERVISOR- [signature]

DATE- 5-19-95

72

6395 Harvested Spinners flasks injected on 9.29.93

2 spinners HTPBS 22 secreted proteins → David
2 " HMSAF 22 Endothelium → Rogers
2 " Rat Stanniocalcin → Pedio
2 " PGF-1S HATCK89 → Rao
1 " HEHBE01-09 → Splicing
1 " Ea

10395 Transfections in CHO cells dhfr-

HUVEC91 → TNF δ pN346

HUVEC91 → TNF δ pCHO-1

see the Sequence Verification Report sheet for more information

TRANSFECTION, SELECTION, CLONING, AMPLIFICATION & SCALE UP of Recombinant CHO.dhfr- Cells.

PHASE I. PROCEDURE:

TRANSFECTION. Co-transfect CHO.dhfr- cells in 35mm ø culture dish using LIPOFECTIN.

PROCEDURE : (T1) Prepare the 35mm ø culture dish of cells the day before doing the transfection so that it is about 80% to 90% confluent.

(T2) Remove the culture medium and wash the plate once with PBS. Add 1ml. of OptiMEM, GIBCO Cat.No.041-01985M without FCS (or 1ml.of culture medium without FCS). Return plate to 37°C until required.

(T3) Prepare the following transfection mix in two separate POLYSTYRENE test tubes, NUNC Cat. No.3-41343A:-

A : 90µl OptiMEM or Culture medium without FBS. 10µl LIPOFECTIN. BRL Cat.No.8292SA.

B : 50µl OptiMEM or Culture medium without FBS. 5µl Plasmid DNA at 1µg per µl. 0·5 1µl Co-transfection DNA at 1 µg per µL (G418 Resistance Plasmid pSV2 Neo).

Mix the contents of tube A with that of tube B and allow to stand for 10 to 15 minutes before addition to cells.

(T4). Remove culture plate from incubator and add the transfection mixture to it dropwise around the plate.

(T5). Return the plate to incubate at 37°C. Shake the plate forward and sideways in order to allow the DNA mixture to be evenly distributed over the cell surface. Incubate the plate for a further 4 to 6 hours After this, add 1ml. of medium containing 10%FBS and 200µg per ml.of Gentamycine. Return the plate to incubate for 24 to 48 hours before harvesting the cells for selection

10.4.95 Harvested CHO cells transfected on 10.3.95 and seeded them in Selective medium

"d

PHASE 2.

SELECTION OF RECOMBINANT CLONES.

PROCEDURE : - (S1) Examine the transfected plate to make sure there is no microbial contamination. The cells should be confluent and in good condition.

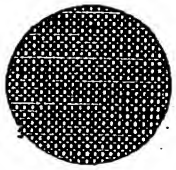
(S2) Remove the culture medium from the transfected plate and wash the monolayer with 2ml. of PBS. Remove the PBS and add 0.5ml.of Trypsin / EDTA GIBCO Cat.No.043-05300. and return to 37°C for 5 minutes.

(S3) Resuspend the detached cells in 5ml of selection medium :

Alpha(-)MEM (without Ribonucleosides & Deoxy ribonucleosides). GIBCO Cat.No.041-02561.
5% Dialysed FBS. GIBCO Cat.No.220-6300AJ.
800 to 1000µg per ml of G418 Sulphate (Geneticin). GIBCO Cat.No.066-1811.
0.01µM Methotrexate (MTX) (+ Aminopterin) SIGMA Cat.No.A-6770. (Prepare a 5 mM stock solution of MTX in Alpha(-)MEM. Sterilize through a 0.2µm. filter. Dispense in 5 ml. aliquots and store in the dark at -20°C).
100 to 200µg per ml of Gentamycine. Amimed Cat.No.4-07F00-H

Resuspend the cells well by gently sucking up and dispensing in order to have a homogeneous cell suspension.

(S4) Dilute this suspension to approximately 45 ml. Dispense 15 ml. of this suspension into each of three 'Hybridoma' cloning plates from GREINER Cat.No.633-160. Take care to fill all the micro-wells and to avoid air bubbles being trapped in them.



'Hybridoma' cloning plate.

(S5) Return the plates to incubate at 37°C for 10 to 14 days. The plates can be examined after a week but colonies are generally to small to be picked at this stage. There should be no need to change the medium over this period unless there are an unusually large number of colonies. If the medium needs to be replaced then use the same medium as above.

Do the following procedure see on page 77

10.5.95 Injections SJ9 cells for virus Stock

HERBE01 - E2 (endometrial tumor specific gene)

HERBE01 - J1

10.5.95 2 seeded today CHO Stammiccalcin 2 clones from T.21.95 in roller bottles in MEM- + 50 µM MTX + 5% dFBS
obs: easy in passaging the cells two times in 1 flask and did work well after 8 days.

10.6.95 Injections Spinners flasks in α-MEM C/FBS

Stanniocalcin	Stc-1-cs	2	spinner
HMSAF22	unknown	2	"
HTPBS22	secreted protein	5	"
FGF-14		2	"
FGF-15		2	"

250 ul of each virus/spinner

10.9.95 Harvested the 5 pg cells Injected on 10.5.95 for virus Stock

HETBE01-D1 blue assay reinfected for 6th

HETBE01-E2

10.10.95 Harvested Spinner flasks injected on 10.6.95

Stanniocalcin	Stc-1-cs	1	spinner to Rajesh
HMSAF22	unknown	2	" to Rajesh
HTPBS22	secreted protein	5	" to David
FGF-14		2	" to Pedro
FGF-15		2	" to Rao

P. Pho

10 10 95 PLAQUE PURIFICATION

DIF Homolog pA2 HARA044
" pA2gP HARA044

Semaphorin homolog pA2 HMBE22 from 9.28.95
" pA2gP HMBE22

HT358 90 D2 unknown from 7.25.95
to obs. this virus had to be plaque
purified again because we finished
big mistake with the 2nd stock plaque ①
that was good in 535 labelling!

obs: no good plaques after 6 days
redone on 10.17.95

10 12 95 Split the Semaphorin clones in two cells
(roller bottles) from 10-5-95

1 split the roller in MEM α - 5% dFBS
+ 25 μM MTX
1:2 → 4 roller bottles after
150 ml of media/roller bottle splitting

10 13 95 Injections Spinner flasks in EX-401 w/o FBS

12 spinners HTPB622 secreted protein
250 ml of virus/spinner

J. Pollo

SUPERVISOR- Kim
DATE- 10/13/95

10/12/95 PLAQUE PURIFICATION

Re-purification of viruses from 10.10.95 because I had no good plaques this time. I injected the Sf9 cells with dilutions from 10^{-4} to 10^{-8}

Unfortunately again I had no good results after 6 days of the purification and I will try from scratch and dilute the virus up to 10^{-12} or 10^{-13}

10/17/95 Harvested Spinners flasks injected on 10.13.95

HiPBS22 12 spinners
(ODGF)

I gave the supernatant to David

J. Mo

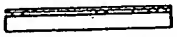
10.18.95 Picking clones of TNF & HIVEN91 CHO cells from 10.4.95

PHASE 1

CLONING

PROCEDURE

(C1) Remove the 'Hybridoma' cloning plates after 10 to 14 days of incubation at 37°C. Suck off the surplus medium with a pasture pipet. Then add 15 ml. of PBS to the dish and swirl around to wash the plate. Remove the excess wash WITHOUT removing any of the liquid from the micro wells.

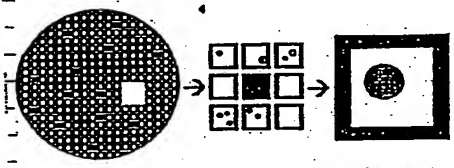


Inverted cloning plate. The surface tension prevents the liquid in the micro-wells from flowing out and prevents the plate from drying out.

(C2) Invert the plate (None of the liquid in the micro wells comes out). Observe the inverted cloning plate under a low power Binocular microscope (NIKON x10 to x30 magnification). This allows you to view a greater area of the plate and to chose colonies much faster.

we do not invert because we don't have this specific Nikon microscope. Instead we look the clones in the normal position and we keep 5 ml of PBS while looking and labeling the clones with a pen.

(C3) With a bit of practice you should be able to pick out wells containing only one clone. Circle or color the well with a NUNC Cryo Pen Cat.No.319993. Choose 24 clones from different parts of the plate.



Most wells containing single clones *Well with a single clone*

(C4) Add 15 ml. of PBS to the plate for a final wash.

(C5) Method 1 : Suck off all the liquid (PBS) form the plate including the liquid in the micro wells.

Add 5 ml. of Trypsin/EDTA to the plate and swirl it around in order to fill all the micro-wells. Tilt the plate and suck off all the excess trypsin. (There is still enough trypsin held in each well to detach the cells). Incubate the plate at 37°C for 5 minutes.

Method 2 : Only suck off the PBS from the micro wells which have been marked. Add 5 µl. of Trypsin/EDTA to the well and incubate at 37°C for 5 minutes. Remove the contents of the well and proceed as directed below in (6).

we do method 2

After removing the clones, add 15 ml. of PBS to the plate, swirl around and suck off all the liquid including that from the micro-wells.

Add 15 ml. of selection medium to the plate and return to incubator. This allows the plate to be used again to pick more clones if necessary.

(C6) Prepare three 24 multi-well plates with 1 ml. of selection medium (as described above but WITHOUT any CHIR) per well.

1	2	3	4	5	6
7	8	9	10	11	12
13	14	15	16	17	18
19	20	21	22	23	24

Number each wells in the three 24 multi-well plates from 1 to 72.

TAKE CARE TO TREAT EACH CLONE SEPARATELY IN ORDER TO AVOID CROSS CONTAMINATION BETWEEN CLONES.

obs: I pick 10 clones of the construct with pCMV-1 vector and 9 clones with the pV346 vector.

I had much more clones with the pCMV-1 vector.

10.19.95 Split Roller bottles from 10.12.95
CHO Stanniocalcin & clones in
MEM α^{+} + 5% FBS
4 split 1 to 4 ⇒ total: 16 roller bottles

10.20.95 Infections Spinners flasks

4 spinners HC FBE49 GP CtGF-3 in α 40% w/o FBS
3 " F6F-15 HA+CR59 "
1 spinner RAT stanniocalcin ① + 1% H.I.FBS
1 " " ② + 1% H.I.FBS

10.24.95 Harvested Spinner flasks infected above

7 sp. CtGF-3 → sup. to Scott
3 " F6F-15 → " to Rao
the spinners of Stanniocalcin media
harvested on 10.25.95

10.25.95 Split Roller bottles from 10.19.95
CHO Stanniocalcin & clones in
MEM α^{+} + 5% FBS
9 split 1 to 4 ⇒ total: 64 roller bottles
obs. I gave 4 L supernatant today
to Alex for purification.

10.26.95 Amplification (Phase 4) of the CHO clones from 10.18.95 TNFβ HUVEC/I

Procedure:

Look the cells and check if the clones are at least 60-80% confluent

Remove the media and make aliquots to check for the best expression

I gave to Dan Bednarik the circled ones

0FBS

100 ul of clone #s

pCHO.1 → (1) (2) (3) (4) (5) (6)

(7)
8 → I changed the media with the same MTX concentration as before = 10nM

5

5

(9)
10 → changed media

pN346 → (a) (b) (d) (e) (f) (i)

e → changed media
g → " "
h → died

Wash the monolayers with 1 ml of PBS
Remove the PBS

Add 250 ul of trypsin-EDTA and incubate for 5 minutes at 37°C

Resuspend the cells adding to them 750 ul of media MEM α⁻ + 5% dialyzed FBS

Prepare the medica with the different concentrations of MTX that you decided to use

a. Dilute 10 µl of MTX (from 5 mM stock solution) in 1 ml of normal + S.I. dFBS

b. take this dilution (1 ml) and add it to 99 ml of medica and you will have the final concentration of 500 nM

Prepare the 6 well dish and add the amount of media with MTX and w/o MTX as below:

MTX concentration	500 nM	normal media
10 nM	40 µl	1.96 ml
30 nM	120 µl	1.88 ml
90 nM	360 µl	1.64 ml

For each clone you will have now 3 wells with 3 different concentrations (10, 30 and 90 nM) Add approximately the same amount of cells in each of the 3 wells (taking care not to mix the different clones)

Incubate at 37°C and look how the cells are growing in the next days

go to

10.27.95 Injections Spinners flasks in CX-40 40FBS

8 spinners HMSAF22

4 " HtPBS22 (CODE F)

10.31.95 Harvested spinners flasks injected virus

8 sp HMSAF22 to Rajan

4 " HtPBS22 (CODE F) to David

10.31.95 Harvested roller bottles (virus) from 10.25.95

I gave the supernatant to Alex ~ 13l

and the media was replaced the same as before.

10.31.95 PLAQUE PURIFICATION

Virus from 10.A.95 that did not work

I diluted the virus from 10^{-1} to 10^{-3}

and used the dilutions 10^{-9} to 10^{-13} in

order to get better plaques

OIC Homolog pA2 HAEA094

pA2GP HAEA094

Semaphorin Homolog pA2 HMBE22

pA2GP HMBE22

A+3SB to

10 3 95 Infections Spinner flasks in α-401 w/o FBS

12 spinners HAQBM60 9-10

cystatin

250 ul of virus/spinner

10 3 95 Harvested the supernatant from roller bottles (Stc 2) from 10.31.95

Alex got ≈ 12 l supernatant
and Ram split 2 roller bottles 1 to 4
to keep as back up in MEM α[+] + 5% FBS

[illegible signature]

FBS 10695 Amplification of CHO cells tNFS HUMECAT

from 10-06-95

Procedure

Look how the cells are growing in the diffent mtx concentrations and if possible make a pool of cells growing good in the 2 highest concentrations (30 and 90 nM)

Remove the supernatant
wash the monolayer with PBS (1 ml)
Remove the PBS and add 250 ul of tripsin and incubate at 37ºC for 5'
Add 250 ul of media and resuspend the cells

Prepare the media with the new concentration of mtx for amplification

Media from 500 nM stock take 9 ml and add it to 41 ml of MEM α + 5% diFBS to have 90 nM and 20 ml of the stock and add it to 30 ml of MEM α + 5% diFBS to have 200 nM mtx

For each clone I have now 3 wells

- MEM α + 5% diFBS for Dan Radioassay analysis
- 90 nM > amplification
- 200 nM

clones in pCHO-1 vector

(1) 10 nM OK 30 and 90 cells died
(2) pool of 30 and 90 nM mtx
(3) " of 30 and 90 nM

(4) I used the cells growing in 30nM
90nM the cells died

(5) pool of 10 and 30nM.

(6) pool of 30 and 90nM.

(7) pool of 30 and 90nM. — clo. clones 8 and 10 I discarded

(9) only 30nM I used

Clones in pN346 vector

(a) pool of 30 and 90nM

(b) only 30nM used

(d) only 90nM used — clo. clones e and g I discarded.

(e) pool of 10 and 30nM

(f) pool of 10 and 30nM

(g) pool of 10 and 30nM

clo: I prepared 96 well for Dan Bednarek in MEM α + 5% diFBS of each clone that I split today for assay

[signature]

10.7.95 Harvested spinner flasks injected on 10.3.95

12 spinners of Cystatin -> sent to Yalong
HAGB460 9.10

10.8.95 transfection in Sf9 cells

HCABA58X (extracellular protein (EGF domain))
plasmid concentration 1.5 ug/ul
procedure like page 1

10.8.95 Infections Sf9 cells with plaque purified virus from 10.31.95

OIF Homolog pA2 HARAO44
OIF " pA2gp HARAO44
Semaphorin homolog pA2 HMBE22
" " pA2gp HMBE22
H735B70 unknown
3 plaques of each but the plaques were too small

11.10.95 Infections Spinners flasks

8 spinners in EX-401 of cystatin HAQBM60.9.10
→ harvested on 11.13.95

2 spinners for testing new media 1S Bac
from Irvine Scientific (w/o FBS)

A2 Regeneration protein HCQB17
ODEF HTPBS22

2 spinners of A2 reg and ODGF in EX-401
w/o FBS to compare how good is the new media
1S Bac (see the gel next page)

11.10.95 Obs: I gave today to Jan Bednarik
the 96 well plate and supernatant
from clones of HOUEO91 in CHO cells
for testing antiviral activity. Obs the cells
were prepared 11.6.95

11.13.95 Harvested Sf9 cells injected with plaque
purified virus from 11.8.95

OIF Homolog PA2 ① ++ Lymphoma homolog ① ++
HARAD44 ② ++ HMAE22 PA2 ② +
③ ++ ③

OIF Homolog PA2SP ① +++ lymphoma homolog ① ++
HARAD44 ② ++ HMBE22 PA2SP ② ++
③ + ③ ++

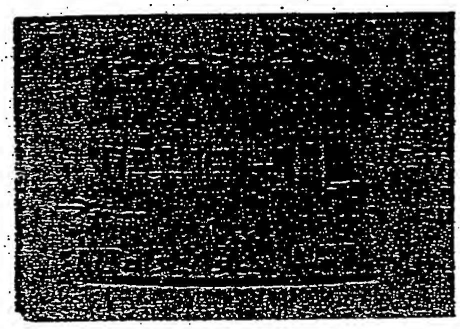
HI35572 ①②③
unknown ++ ++ ++

13B95 Transfected transfection from 11B93

11CA3A55x (extracellular protein / EGF domain)

blue assay + but not so strong

11B95 Machine Sequence 11B93



1,2 Control OK [illegible] 101139

3 CDEF (EY-401) we can see the band below 55

4 CDEF (15 BAC) we can not see the band below 55

5 A_2 Regulation protein (EY-401) we can see the band

6 A_2 Regulation protein (15 BAC) we can see the band

Conclusion: the 15 Bac media seems to be OK but as we can see CDEF was not secreted so we better try to adapt the cells in spinners before infection to make our final conclusion!

J. N. B.

11.15.95 Amplification of CHO cells TNF / [illegible]
from 10.6.95

Clones in pCMO-1 vector

#	α	90nM	200nM	comments
1	OK	Died	Died	split 2 cells in 30nM mtx
2	OK	few clones	Died	split some cells again in 90nM
3	OK	very good	good	pool of 90 and 200 in 100nM
4	OK	few clones fighting	Died	split 90nM in 90nM
5	OK	few clones fighting	Died	split 90nM in 90nM
6	OK	very good	very good	pool of 90 and 200 in 100nM
7	OK	few clones	Died	split 90nM in 90nM
9	OK	few clones	Died	split 90nM in 90nM

Clones in pN346 vector

#	α	90nM	200nM	comments
a	OK	good	few clones fighting	pool of 90 and 200 in 100nM
b	OK	few clones	" " "	pool of 90 and 200 in 100nM
d	OK	good	few clones	"
e	OK	clones fighting	some cells	"
f	OK	few clones	died	pool of 90nM in 100nM mtx
i	OK	good	some cells	pool of 90 and 200 in 100nM

the media was prepared like page 83
and the procedure for the cells also
according to Dr [illegible] the clones # 3, 5, 7 and
b, e showed some antiviral activity

11.15.95 PLAQUE PURIFICATION

HCABAS8X (extracellular protein / EGF domain)

from 11.13.95

11.16.95 Injections Spinners flasks in EX-401 w/o FBS

11 spinners of Cystatin HAQBM609-10

11.20.95 Harvested spinners injected above

Supernatant to Yuling

11.21.95 Amplification of CHO cells TNF-γ HUVEO9 from 10.15.95

clones in pcDNA vector

#	MTX Concentration	Comments
3	100nM MTX	excellent, confluent > pool 200, 300 nM MTX
3	200nM	" "
6	100nM	very good " > pool 200, 300 nM
6	200nM	" "
1	30nM	good → split 50nM and 100nM
2	90nM	good → " 150nM and 100nM
4	90nM	few clones fighting > change media to 100nM
	90nM	
5	90nM	some cells fighting > " " 100nM
	90nM	
9	90nM	some clone → " " 100nM
9	90nM	" → " 100nM

clones in pcDNA vector ⟹

pN346 vector

m+y

#	Concentration	Comments
a	100 nM 100 nM	v.good 90% confluent > pool 100:200 v.good " > and seed in flask
b	100 nM 100 nM	few clones > change media to " 100 nM
d	100 nM 100 nM	excellent > pool 100:200 flask "
e	100 nM 100 nM	few clones > Change media fighting > to 100 nM
f	100 nM 100 nM	" > change media to 100 nM
i	100 nM 100 nM	few clones > change media " to 100 nM

Media preparation:

From 500 nM Stock to prepare:

100 nM	5 ml of the stock	into 20 ml	of MEM-
200 nM	10 ml "	" 15 ml	"
300 nM	15 ml "	" 10 ml	"
50 nM	0.2 ml	into 1.8 ml	"
150 nM	0.6 ml	" 1.4 ml	

[signature]

11.20.95 Infections Spinners flasks in ex-401 (11/17/95)

8 spinners HMSAF22

2 " 6P-F spondin HESA 920

2 " A2-F spondin HESA 920

amount of virus 180 ul / spinner
due to the Holidays we used less this time!

11.20.95 Infections Sf9 cells with plaque purified virus from 11-15-95

HCABA58X (extracellular protein / EGF domain)
3 plaques

11.21.95 Harvested Sf9 cells infected above

			blot assay
HCABA58X	①		+++
"	②		++
"	③		+

11.24.95 Harvested Spinner flasks infected on 11.22.95

8 spinners HMSAF22 → sup. to Stev

2 " 6P-F spondin } HESA 92
2 " A2-F spondin }
"

sup. and cells

11/28/95 Amplification of CHO cells + MF-2 HUV-091 from 11.21.95

Clones in pcDNA-1 vector

#	MTX concentration	comments
3	200 nM	Very good and confluent
3	300 nM	split in 300, 400 nM MTX → bigger flask
6	200 nM	good, 80% confluent cells
6	300 nM	floating a little bit, split to 300, 400
1	2-α	OK but I won't use it anymore
1	50 nM	many clones, split to 50 nM in + flask
2	2-	OK but I won't use it anymore
2	50 nM	cells look OK; confluent split to 50 nM to + flask
4	100 nM	good clones, make a pool
4	100 nM	split to 100, 150 nM in + flask
5	100 nM	very good clones, make a
5	100 nM	pool and split to 100 nM + flask
7	100 nM	overconfluent, split to 100 and 150 nM + flask
9	100 nM	→ 80% confluent, split to 100 and 150 in + flask

Media preparation from 500nM MTX stock

	MTX stock	MEM 1-
50 nM	12.5 ml	22.5 ml
100 nM	10 ml	40 ml
150 nM	15 ml	35 ml
200 nM	20 ml	30 ml
300 nM	30 ml	20 ml
400 nM	40 ml	10 ml

Clones in pRN346 vector

#	MTX concentration	Comment
a	100 nM	good split to bigger
a	200 nM	very good flask, 200, 300 nM
d	100 nM	confluent but the cells have
d	200 nM	different shape. Pool and split in bigger flask
b	100	no clones make a pool
b	100	and split in 100, 200 (+flask)
e	100	good clones, the same
e	100	like b (100, 200)
f	100	few good clones, pool
f	100	and split in [illegible] - flask
λ	100	good clones the same
λ	100	like b (100, 200)

11 28 95 Injection Sf9 cells for virus Stock

HMQBE22 (1) [illegible] Semaphorin homolog from 11.3.95
obs: this clone is not full length

obs: HARAO44 PA2 → Dif Homolog
" PA2gp was not expressed (S^{35} labeling)

HMQBE22 A2 was expressed but not secreted
and HMQBE22 A2gp was secreted [illegible]

11/28/95

H135S7C plaques 4,5,6 we could [illegible]
[illegible]

11 30 95 Injections spinner flasks (ex-401 w/o FBS)

12 spinner A2HNGF HHPSH35

250 ul of virus/spinner

12 1 95 transfections in Sf9 cells

DNA:

pA2 HSAAU35 (X-CSFR) 0.5ug/ul → mini prep!!!

pA2 HBJEL88 (thymus specific unknown) 1 ug/ul

procedure see page 1

12 4 95 Harvested Sf9 infected on 11.28.95 for virus stock

HMEBE22(1) pA2GP semaphorin homolog

blue assay positive

12 4 95 Freezing of TNF clones (HUVE091) in CHO cells from 11.28.95

Freezing media: 5% DMSO, 10% FBS MEM α+

clones #: 3 300nM MTX > cells looked very good
3 400nM MTX > pCHO-1 vector

6 300nM MTX > 90% confluent cells looked
6 400nM MTX > OK pCHO-1 vector

d 150 nM 8 st. confluent, cells looked ok pN346 vector

a 200 nM
a 300 nM } Cells looked good pN346 vector

2 prepared 3 vials with 1 ml of each clone and the cells are resistant up to the concentration of MTX indicated.

12 5 95 Harvested spinner flasks inocked on 11 3 95.

12 spinners A2 TNF HYPS35 supernatant to Scott

12 6 95 Split TNF clones (HUVEC91) CHO cells from 11 28 95 in MEM 1+ for freezing next week

pCho-1

#	MTX concentration (growing)
1	50 nM → many nice clones
2	50 → many single cells (??)
4	100 → many clones fighting
4	150 → " " "
5	100 → few good clones
7	100 → nice cells 50% confluent
7	150 → cells growing good
9	100 → 95% confluent - good cells
9	150 → 50% " "

pN346 vector

#	growing MTX concentration
e	100 nM very good cells 85% confluent
e	200 very good clones 60%
d	100 nM 90% confluent nice cells
i	100 nM many single cells (??)
i	200 nM 100% confluent, different shape
h	100 nM excellent 95% confluent
h	200 nM nice clones 65% confluent

12/6/95 harvested transfection in Sf9 cells from 12/1/95

HB3EL88 thymus specific unknown
new assay was positive

12/6/95 PLAQUE PURIFICATION

HB3EL88 thymus specific unknown
procedure see page I

12/7/95 Infections spinners flasks in Ex-401 w/o FBS

4 spinners A2 LINK 36 A 1 HCEDD36A

4 " GP-F spondin HESA970

4 " GP 16i2 LinK 73 HTECD31

11/95 Harvested transfection in 175 cells from 12.7.95

Obs. Blue assay was weak

HSAAV35 (X-CSFR)

12/95 Freezing of TNF clones (HAVEC91) in CHO cells from 12.5.95

Obs: all clones frozen today were good and confluent and I made 3 vials of each

freezing media: 10% FBS, 5% DMSO in MEM α

PCHO-1 vector:

1	amplified up to 50mM MTX
9	100 nM
9	150 nM
7	100 nM

pSV346 vector:

a	100 nM
b	100 nM
b	200 nM
c	200 nM
e	100 nM

12/95 Harvested spinners flasks injection 12.7.95

4 spinners A2 UNK 36 A-1 HCEDD36A super to Stephan

4 " GP-F spondin HESA 320 super to Pedro

4 " GP FGF 2 like 7 1 HTEC931 " to David

12.12.95 Freezing of TRP clones (new ???) in CHO cells from 12.5.95

On all clones (two ext) frozen today in general looked good but some were less confluent than others that's why I prepared 3 or 2 vials of each.

pN346 vector

2 amplified up to 200 nM MTX 3 vials

1 " 100 nM 2 vials

p CHO-1 vector

4	100 nM	3 vials
4	150 nM	3 vials
2	50 nM	3 vials
5	100 nM	2 vials
7	150 nM	3 vials

12.14.95 Infection Sf9 cells with plaque purified virus from 12.6.95

HB4CL88 thymus specific gene
3 plaques

12.14.95 Infection Sf9 cells for virus stock from 11.28.95

HCABIA58 (3) extracellular protein
EGF domain

12M95 PLAQUE PURIFICATION

from 12.7.95

HSAAU35 (X-CSFR)

procedure: like page 1

see 12.19.95

12.14.95 Infections Spinners flasks in EX40.0 cells

HTECD31 +6F2 like gp 6 spinners

HCABAS8(3) extracellular protein EGF domain 2 spinners

gp HMRSM23 4 spinners

250 ul of viral/spinner

Harvested by Nam on 12.18.95

12.19.95 Harvested (by Nam) Sf9 cells infected on 12.14.95 with plaque purified virus

HBJEL85 thymus specific gene 6 (3)

12.19.95 Harvested virus stock infected on 12.14.95 by Nam named the stock

HCABAS8(3) is extracellular protein EGF domain

SUPERVISOR: [illegible]

DATE: 12/19/95

12.18.95 PLAQUE PURIFICATION (done by Nam and the uninfected data the Sf9 cells with the plaques!

HSAAU35 (X-CSFR)

1996

1.1996 Infections Spinner flasks in EX-401 w/o FBS

2 spinners HCABA58 (extracellular protein Elaf domain)

2 - " TGF α active domain (HTECD31)

5 " HNGF (HHPSH35)

1.1996 Thawing of CHO clones of TNF (HUVE091) in pCHO-1 vector

clone numbers	concentration of MTX already resistant:	freeze date:
4	100 nM	12.12.95
4	150 nM	12.12.95
2	50 nM	12.12.95
5	100 nM	12.12.95
1	50 nM	12.8.95
9	100 nM	12.8.95
9	150 nM	12.8.95
7	100 nM	12.8.95
3	300 nM	12.4.95
3	400 nM	12.4.95
6	300 nM	12.4.95
6	400 nM	12.4.95

All cells were seeded in MEMα+ + 5% FBS

1 2 96 thawing new crodhp D944

Concentration / vial : 1 x10⁶ cells/ml
12.20.95 - 4
frozen by Jinli
the cells were seeded in MEM+ +5% FBS

1 23 96 Infections Sf9 cells for virus Stock

HBJEL88 (2) thymus specific - pA2
50 ul of virus / t 225 t flask

1 23 96 Harvested Spinners flasks infected on 1.18.96

2 spinners HCABA53 → Stephen
2 " TGF α active domain → David
5 " HNbF → Scott

1 23 96 Transfections in Sf9 cells

HTOJK64 (ODP AC) pA2 0.39 ug/ul (Ping Feng / P. Dillon)
HETG170 (ESBF II) pA2 0.6 ug/ul (Guo-liang Yu / Jian Ni)
HFCB502 (Cystatin II) pA2GP 1 ug/ul (Guo-liang Yu / Jian Ni)

procedure like page 1

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1.24.96 Amplification of TNF (HUVEC5!) in CHO cells

thawed on 1.22.96

	clone #	amplified up to	split to
	9	100 nM MTX	200 nM MTX
	9	150	300
	7	150	300 nM
	1	50	100 nM
	4	100	200 nM
	4	150	300 nM

Media: from 500 nM MTX stock } dilutions:
100 nM: 2 ml of the stock + 8 ml of α- } 10 µl + 1 ml + 99 ml = 500 nM
200 nM: 20 ml " 30 ml of α-
300 nM: 30 ml " 20 ml of α-

In general all cells looked good and had 70 to 95% confluency

the other clones #s 2, 3 and 6 probably will be amplified tomorrow 1.25.96

	clone #	amplified up to	split to
1.25.96	2	50 nM	100 nM
	3	400	500
	3	300	400
	6	300	400
	6	400	500
	7	100	200

1 26 96 Injections Spinners flasks in EX-401 W/0 FBS

obs: The cells were prepared the day before

3 spinners HB9EL88 Thymus specific
2 " HHWAF38 EMAP II F.2
2 " HTPBS22 ODGF OAP + H. H.I.F.B.S
3 " HRDCD54 Myelin oligod.
2 " HSAAU35 CSF-R like
400 ul of virus/spinner

obs. Harvested on 1.31.96

1 26 96 Transfections in CHO dhfr- D944

HTPBS22 (appC102) ODGF pC-1 vector
DNA ≈: 2.5 ug/ul - Pingfeng/Patterson

HWFBD68 CKB-1 pC-1 vector
DNA ≈: 0.6 ug/ul - Haodong Li

procedure: see page 72

1 26 96 Harvested Sf9 cells infected
on 1.23.96 for virus stock

HB9EL88 (2) thymus specific
p1A2 [label: A2 Thymus spec #2 HB9EL88 HG12580 SI 1/26/96]

blue assay: ++

1 2996 Selection of Recombinant clones (CHO dhfr-9944) transfected on 1.26.96

HTPBS22 (appC102) pC-1 vector
HWEBD628 (CKB-3) pC-1 vector

procedure and media preparation see page 73

1 2996 Harvested transfections in Sfg from 1.23.96

	blue assay
HTO3K64 (OAP ΔE) pA2	+++
HET6170 (ESBF II) pA2	+++
HFCB502 (Elplatin II) pA260	+++

1 2996 Amplification of TNF CHO clones (HWVC691) from 1.24.96

clone #	amplified up to	split to	
5	200nM MTX	300nM MTX	clone (lighting 687)
9	300	400	
3	100	200	
9	200	300	
9	300	400	
4	200	300	
4	300	400	

clones from p. 61

09441 | 1.30.96 Amplification of tNF CHO clones (CHUV2291) from 1.25.96

Clone #	[MTX]	split to
2	100 nM MTX	200 nM MTX
3	500	600
3	400	500
4	200	300
6	400	500
6	500	600

the clones are growing pretty good

2.1.96 PLAQUE PURIFICATION (dw did not work)

H10JK64 (DAP ΔC) pA2

HET6170 (ESBP II) pA2 — 1 diluted from 10^{-1} to 10^{-10}

HFCBSO2 (Cystatin II / SAL-4.2456 / association) pA2 6P

HOUCK17 Muth-1 A2 } from tim

HOUCK17 Muth-1 gP } from tim

procedure see page 1

2.1.96 Amplification of tNF CHO clone (CHUV2291) from 1.30.96

4917

	split to
# 3 (500)	600 nM MTX
# 3 (600)	700 nM MTX

this clone is growing fa

SUPERVISOR [signature]

DATE 2/1/96

106

2.29.96 Injections Spinners flasks in DX-401 with MTX

1 spinner HTA3R03 (EMAP II)
6 spinners HB9EL88 (thymus specific)
5 " L6IF
300 ml ea vented spinner

2.5.96 Amplification of CHO clones TAF (HUVEC 1)
from 1.29 and 1.30.96

Clone #	MTX resistance	split to:
3	700	Kept
3	600	Kept
2	200 good, confluent	300 nM MTX
2	300 " 70% "	400
6	600 " 80% "	700
6	500 " 80% "	600
5	300 few clones	300
1	200 good, confluent	300
7	400 " "	500
9	400 " "	500
9	300 OK 80%	400
4	400 OK 70%	500
4	300 good, 80% Conf.	400

media: from 1000 nM MTX stock

300 nM	→ 6 ml	→ 14 ml α-MEM + dFBS
400	→ 8 ml	→ 12 ml
500	→ 10 ml	→ 10 ml
600	→ 3 ml	→ 2 ml
700	→ 2.5 ml	→ 1.5 ml

2.6.96 Harvested spinners flasks injected on 2.2.96

1 sp HTABK03 (EMAP III) supe to Jeffrey
6 sp HB9EL88 supe to Yuling
5 sp A61F supe to Padia

12.7.96 PLAQUE PURIFICATION (repetition from 2.F96)

HTOJK64 (DAP) pA2 supe De
HETG170 (ESBF II) pA2
HFCBS02 (Cystatin II) A2 gp
HOUCK17 Meth-1 A2
HOUCK17 Meth-1 gp } from Tim

I made dilutions from 10^{-1} to 10^{-13}

Procedure like page I

2.7.96 Transfection in Sf9 cells

HTOAU65 TNFR (soluble)
pA2 gp Project code H602800

Construct from Jian Ni

DNA concentration: 0.4 ug/ul

2.9.96 Injections Spinner flasks in EX-401 w/o FBS

5 spinners HRBC 254
2 spinners HtABKO3

4 spinners HBJEC88
1 spinner HBJEC88 + 1% HI-FBS

amount of virus / spinner : 400 ul

2.9.96 Amplification of TNF clones in CHO cells from 2.5.96

clone #

1 (300)	very good	split to 400 nM MTX
5 (400)	not good, few cells growing - keep	
9 (500)	good	split to 600 nM MTX
9 (500)	good	" to 600 nM
9 (400)	very good	" to 500 nM
4 (500)	very good	" to 600 nM
4 (400)	very good	" to 500 nM
3 (600)	good	" to 700 nM > made a pool
3 (700)	"	" to 700 nM > because I had less cells
2 (300)	very good	" to 400 nM
6 (700)	"	" to 800 nM
6 (600)	"	" to 700 nM
7 (400)	few cells growing - keep	

Media from 1000 nM stock
↓

600 nM → 9 ml + 6 ml mem α + 5% diff
500 nM → 5 ml + 5 ml
400 nM → 4 ml + 6 ml
700 nM → 7 ml + 3 ml
800 nM → 4 ml + 1 ml

2.13.96 Harvested spinners flasks injected on 2.9.96

5 sp. HRDCD54 supe to Arvind
2 sp. HABK03 supe and cells to Jeff
4 sp. HB9EL88 → supe to Yuling
1 sp. HB9AL8 + PBS

2.14.96 Harvested transfection in Sf9 cells from 2.7.96

HTOAU65 TNF R (soluble)

pA2 GP Project code: HL0232C0

2.14.96 Picking clones from CHO cells dhfr- D944 in selective media from 1.29.96

HTPS22 (sop C102) pC-1 vector
HWEBD68 (CKB-1) pC-1 vector

We have many clones of each construct this time!

Procedure was a little bit changed this time:

take the hybridoma plate, discard the media and wash twice with PBS
Remove the PBS and add 5ml of trypsin
incubate at 37°C for 5 minutes
with your scope inside the hood examine your best clones and with a bit of practice pick one by one and seed in MEM α+ + 5% FBS (if you want to test for antiviral activity) or in selective media + MTX (20nM) + PS
→ MEM α- + 5% dFBS

In both cases we seeded this time in
96 well dish) 1 ml of media/well
- Incubate the cells at 37°c and look in 3 or 4 days how the clones are growing.

2 pick this time 48 clones of OAP
48 clones of CKB-1

and as a back up I changed the
on 2.15.96 media of 1 hybridoma plate of each
gene and incubated 37°c for a week or so.
I used 20 mM MTX instead of 10 mM

2.15.96 Amplification of +NF clones in CHO cells
from 2.9.96

Clone 1

3 (700) bec confluent, fighting a bit, split to 800 nM MTX
2 (400) confluent, very good, split to 800 nM

6 (700) > bec confluent but fighting for this resistance,
6 (800) split to the same concentration of MTX

media: 5 (400) cells died

bec confluent 7 (400) not confluent, cells look ok, split to 800 nM
600: 6 ml 4 ml 1 (400) v. good " " to 800 nM
700: 14 ml 16 ml 9 (600) ok split to 800 nM
800: 8 ml 12 ml 9 (500) > very good split to 600 and 800 nM
9 (600)

9 (800) v good -> split to 600 nM
4 (600) v. good " 700 nM

2.16.96 Injections Spinners flasks in Ex-401 cells

2 spinners thymus specific HBSEL88
5 " A2 T6FL full length HTECD31 (??)
5 " HRDCA54

2.16.96 Injections Sf9 cells with plaque purified viruses from 2.7.96

HTO9G64 (OAP) pA2
HETEI70 (ESBF II) pA2
HFCBS02 (Cystatin II) AsgP } from tim
HOUCK17 Meth-1 A2
HOUCK17 Meth-1 A2 Sp

2.16.96 Injection Sf9 cells for Virus Stock
HSABU35 (X-CSER) #3
50 ul of virus / 1 flask

2.20.96 Harvested Spinners flasks injected on 2.16.96

2 sp HBSEL88 → to Yuling
5 sp A2 T6FL → to David
5 sp HRDCA54 → to Arvind

obs: this was the day of our moving to 9410

2.21.96 Harvested Sf9 cells injected on 2.16.96 for virus stock

HSAAU35 (GCSF-R) #3 — A2 GX-CSF Rec #3 HSAAU35 ... 2/21/96

blue assay was good.

2.21.96 Harvested Sf9 cells injected on 2.16.96 with plaque purified virus:

HEO9K64 OAP pA2

HE+6I7O ESBPII pA2

HFCBS02 Cytokine I A gp

HOUCK17 Meth-1 A2 } for tim

HOUCK17 Meth-1 A9 6P

blue assay was good for all

2.22.96 Changed the media of CKB-5 clone #HWFBD68 and HPBS22 (22p clo2) OAP in CHO cells from 2.14.96 and TNF clones from 2.13.96

obs: I used MEM α+ +5% FBS in order to give samples for assays.

HPBS22 - OAP → I gave to Dan Bednaux clones #s 1 to 48
49 CHO dhfr- 7944 supernatant
50 MEM α+ + 5% diFBS

HWFBD68 CKB-1 I gave to Erik for the Biacore. clones #s 1 to 48
49 CHO 7944 supernatant

Results for the antiviral assay Dan did not detect antiviral activity.

for the Biacore assays see the results on 2.2.9c

2.2.96 Amplification of CHO clones: OAP, CKB-1, TNF

1) OAP #+pB522 - split all 48 clones in 20 mM MES

2) CKB-1 #WFBD68 - split all 48 clones in 20 mM MES

3) TNF clones #1:VED91

clone #

968

4(600)	split to	700	in MES
4(700)	"	800	
9(700)	"	800	all clones looked
9(600)	"	700	in general good!
7(500)	"	600	media from
7(700)	"	800	pD1000 in MES
1(500)	"	600	600 nM: 9 ml + 6 ml MES mix
			700 nM: 7 ml + 3 ml
			800 nM: 6 ml + 4 ml
2(500)	"	600	900 nM: 9 ml + 1 ml
3(900)	"	900	
6(700)	"	800	
6(600)	"	900	

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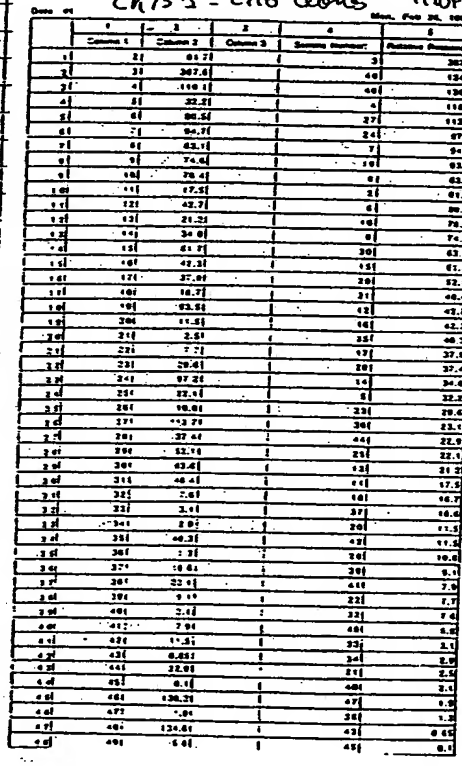
22796 Amplification of CHO clones: OAP, CKB1 from 2.23.96

CKB1: Results from the Biacore

CKB1 - CHO clones HWFB968



clones that we pick to amplify:

1s 3, 48, 46 seeded in 20, 30 and 50 mM MTX

clones that we pick to freeze as back up:

1s 4, 27, 24 seeded in 20 and 40 mM MTX

OAP: HTPB522

split all clones in 40 mM MTX for amplification and 20 mM MTX for 535. labelling on 3.1.96

2.27.96 thawed new Sf9 cells

1 Vial P. 31-32 seeded in Grace 10% HIFBS
1 Vial P. 31-32 " in EX-401 2% FBS

2.28.96 transfections in Sf9 cells

HHPEW62 from Yi. Li pA2 DNA ≈ [0.5 ug/ul]
HSVAJ86506 from John Greene 16F-1 mature peptide fused to gp signal peptide. DNA ≈ [1 ug/ul]

Procedure like page 7

2.28.96 Amplification of TNF-hulEDI CHO clones from 2.23.96

clone #s

4 (700)	split to	800 nM MTX	
4 (800)		900	
9 (700)		800	
9 (800)		900	
2 (600)		700	all clones looked
1 (600)		700	OK
7 (600)		700	more than 80% confluency
7 (800)		900	
3 (900)		1000	
6 (800)		900	> those clones the cells
6 (900)		1000	looked a bit fighting for the resistance

from 1000 uM MTX stock

Media:
800 nM → 8 ml → 2 ml
900 nM → 18 ml → 2 ml
700 nM → 10.5 ml → 4.5 ml

SUPERVISOR- [signature]
DATE- 2/29/96

3.1.96 Infections spinner flasks in EX 401 w/c FBS

4 spinners VEGF-2 HOSBD47 0400
4 " VEGF-3 HMWCF06 0460co
2 " A35

1 " HOUCK17 Mth-1 (3) Ax
1 " HOUCK17 Mth-1 (3) GP

400 ul of virus/spinner

3.1.96 S^{35} Labelling of DAP. CHO clones from 2.27.96

obs: Reiner labelled the cells for me

The cells were kept in the same media 2mM MTX, 5% dFBS in MEM α- and the radioactive cys, (2 ul/well) was added.

100 ul of the cys was diluted in 900 ul of the media and the amount of radioactive material/well was ≈ 20 uCi.

3.4.96 Kathy prepared the gel and loaded the samples for electrophoresis.

3.4.96 Amplification of CKβΔ clones from 2.27.96 and CAP clones.

CKB1

clone #s (for amplification)

3
46
48

I discarded the 20nM and seeded the cells growing in 30 nM in 40nM and the cells growing in 50nM in 60nM

clone #s (for freezing)

4
24
37

I discarded the cells growing in 30 nM of #4 and #37 and seeded the cells growing in 40 nM in 50 nM.

#24 I made a pool of 30 an 40 nM and split in 50 nM because I had less cells

All clones looked in general good!

CAP: htPBS22 (48 clones)

The cells of clone #20 died and was discarded

All the rest I split in 60nM MTX and in general all clones looked good!

1well

[illegible]

3596 Harvested Spinners injected on 3.1.96

The supe of VEGF-2, VEGF-3 and A35 I gave to Scott

The supe of Meth-1 ③ I gave to Alex

3596 Harvested the transfections from 2.28.96

Obs: HSVA 786 506 gp was contaminated/ probably in the prep! Lon 3.4.96
↳ to be redone

HHPEN62 was not contaminated but the blue assay was a little bit weak but positive!

3696 Amplification of tnf clones from 2.28.96 ↳ HHPEN!

clone #s

7 (700) discarded

7 (900) split in MEM-, 1, 3 and 5 μM Lit X

3 (1000 nM) " in " , 2, 4 and 6 μM

6 (900) discarded

6 (1000 nM) split in MEM-, 2, 4 and 6 μM

1 (700 nM) " in " , 800 nM, 1 and 2 μM

2 (700 nM) " in " , " , 1 and 2 μM

9 (800) discarded

9 (900) split in MEM2⁻, 1, 3 and 5 μM

4 (800) discarded

4 (900) split in MEM2⁻, 1, 3 and 5 μM

3.6.96 PLAQUE PURIFICATION

A; HHPEN62 (from 3.5.96)

HTOAU65 gp TNF R soluble (from 2.14.96)

A2 IL-10 homol. HMQBM23 → from Ram

GP IL-10 " HMQBM23

3.8.96 Injections Spinner flasks in cross works

2 spinners T6F2 HTECD31

6 " HCEDD36 FSP-2

3.8.96 Transfection in Sf9 cells

HSVA 186 S06 gP from John Greene

= 16F1 mature peptide fused to gP signal peptide

Redone for the second time

see 3.5.96

120

3.8.96 Amplification of CHO D944 clones

CK/B1 and OAP from 3.4.96

CK/B1 (HWFBD68).

clones #s (for amplification)

3, 46, 48 growing before in 40 and 60 nM all looked good.

1 split all 3 clones in MEM- + 5% diFBS, 50 nM, 100 nM, 200 nM, 500 nM in a 6 well dish.

clones #s growing before in 50 nM. 1 split in MEM- 4, 24, 27 + 5% FBS + 60 nM MTX

OAP (HTPB328)

clones #s 4 and 6 growing in 60 nM confluent, 1 split in MEM-, 50 nM and 100 nM (Rivell)

Obs: Results of the 535 labelling from 3.4.96

clones #s 1, 4, 6, 26 and 44 showed good production

#s 4 and 44 showed even better production of OAP in 14 hours of exposure

On 3.8.96 I amplified the clones #s 4 and 6 in MEM- + 5% diFBS, 50 nM and 100 nM (Rivell)

3/11/96 Amplification of CHO D944 clones from 3.8.96

CK/B1 (HWFB168)

clone #5

48 growing before in 2- 50, 100, 200, 500 nM

split in 2-, 200, 300, 400 (100 of 50/100, 200)

the cells of 500 nM died

\# 3 growing in α⁻, 50, 100, 200, 500 nM
split in α⁻, 200, 300, 500 nM (pool of 100, to 500)

\# 46 growing in α⁻, 50, 100, 200, 500 nM
split in α⁻, 200, 300, 500 (pool of 100, 200, 500)

\#s 4, 24, 27 growing in 60 nM MTX & split in MEM α+ + 5% FBS in order to expand the cells fast for freezing as a back up.

In general the clones of CKBs looked good!

ODP (#PBS22) from 3.8.96

Clones #s

3.9.96 { 4 growing in α⁻, 50, 100 nM
split in α⁻, 100, 200, 300 (Pool of 50, 100 nM)

6 growing in α⁻, 50, 100 nM
split in α⁻, 100, 200, 300 (pool of 50, 100 nM) }

(Wed) 1 growing in 60 nM MTX
26 " " " → in 24 well
8.96 44 " " "

9 split in α⁻, 100 nM # 1
α⁻, 100, 200 nM # 26 > 6 well
α⁻, 100, 200 nM # 44
I did not use this concentration only 100 nM!

3.12.96 Harvested Spinner flasks: infected on 5.3.96

2 spinners +GFL → supernatant to [illegible]

6 " ESP-2 → " to Stephan

3.13.96 Amplification of CHO TNF clones HIV-E091 from 3.6.96

Obs: in 3.11.96 I changed the medium of all clones (MEM α+ 5% FBS) to recover the cells a lit bit because it was not so good and in 3.13.96 the cells looked good again I started to split.

Clone #5

2 met the cells growing in 2 μM in 1, 2, 4, 6 μM

9 " " " " in 5 μM in 1, 5, 7, 10 μM

4 " " " " in 5 μM in 5, 7, 10 μM

6 " " " " in 6 μM in 6, 8, 10 μM

3 " " " " in 6 μM in 6, 8, 10 μM

3 " " " " in 2 μM in 1, 2, 4, 6 μM

3 " " " " in 5 μM in 1, 5, 7, 10 μM

[illegible signature]

3.B96 Infection Sf9 cells with plaque purified virus from 3.6.96

A2 HHPEN62

H10 AU65 gp TNFR soluble

3.M96 Harvested transfection in Sf9 cells from 3.8.96

HSVA 386 506 gp

IGF-1 mature peptide fused to gp signal peptide

3.M96 Freezing of CHO clones CKB-1 HWPBD68 from 3.M.96

The clones are resistant up to 60nM MTX

Freezing media: MEMα+, 5% DMSO, 10% FBS

clones frozen #4, 24, 27

3 vials of each

Overnight at -70°C and after at -140°C

cell density/vial - 3x10⁶ cells/ml